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Aspartate-derived Amino Acid Biosynthesis in *Arabidopsis thaliana*

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The aspartate-derived amino acid pathway in plants leads to the biosynthesis of lysine, methionine, threonine, and isoleucine. These four amino acids are essential in the diets of humans and other animals, but are present in growth-limiting quantities in some of the world's major food crops. Genetic and biochemical approaches have been used for the functional analysis of almost all *Arabidopsis thaliana* enzymes involved in aspartate-derived amino acid biosynthesis. The branch-point enzymes aspartate kinase, dihydrodipicolinate synthase, homoserine dehydrogenase, cystathionine gamma synthase, threonine synthase, and threonine deaminase contain well-studied sites for allosteric regulation by pathway products and other plant metabolites. In contrast, relatively little is known about the transcriptional regulation of amino acid biosynthesis and the mechanisms that are used to balance aspartate-derived amino acid biosynthesis with other plant metabolic needs. The aspartate-derived amino acid pathway provides excellent examples of basic research conducted with *A. thaliana* that has been used to improve the nutritional quality of crop plants, in particular to increase the accumulation of lysine in maize and methionine in potatoes.

INTRODUCTION

Lysine, methionine, threonine, and isoleucine (Figure 1) are synthesized via a branched pathway from aspartate (Figure 2), and hence are commonly called the aspartate-derived amino acids. Whereas most plants, bacteria, and fungi have enzymes for the biosynthesis of these amino acids, animals do not. Therefore, as a group, the aspartate-derived amino acids constitute four of the eight essential amino acids that humans and other animals must obtain in their diets or, in some cases, from other sources such as rumen microflora (Bach et al., 2005) or endosymbiotic bacteria (Douglas, 1998).

Research interest in the biosynthesis of aspartate-derived amino acids is driven in part by their economic value. Major field crops, which either directly or indirectly (as animal feed) make up the majority of the diets of most human populations, are deficient in one or more of the aspartate-derived amino acids. These deficiencies include lysine and threonine in cereals (Debadov, 2003; Pfefferle et al., 2003), methionine and threonine in legumes (Muntz et al., 1998), as well as methionine and isoleucine in potatoes (Stiller et al., 2007). Amino acids that are produced synthetically or by fermentation are often added to animal feed to improve its nutritive value. The world-wide cost of these supplemented amino acids is considerable, estimated at several billion dollars annually (Mueller and Huebner, 2003). As an alternate approach, plant breeding and agricultural biotechnology methods are being used to increase the essential amino acid content of crop plants through targeted manipulation of the aspartate-derived amino acid biosynthetic pathway. This would provide added value for farmers and seed companies, and could also lead to improved human nutrition in parts of the world where a single plant species, for instance rice or maize, makes up the majority of the diet.

An additional practical interest in studying amino acid biosynthesis pathways comes from their role as herbicide targets. The fundamental requirement of amino acids for plant survival, as well as the absence of essential amino acid biosynthesis in humans and other animals, makes the aspartate-derived amino acid pathway an attractive target for herbicide development. For instance, acetolactate synthase, an enzyme in the biosynthetic pathway leading from threonine to isoleucine, is the target of several classes of economically important herbicides, including sulfonylureas, imidazolinones, triazolopyrimidines, and pyrimidinyl oxybenzoates (Mourad and King, 1992; Ott et al., 1996).

Although biosynthesis of aspartate-derived amino acids has been studied in several plant species, many of the recent advances in this field have come from research conducted with *Arabidopsis thaliana.* The well-developed genetic resources available for this model plant have led to numerous new discoveries, including not only previously unknown biosynthetic enzymes, but also novel regulatory mechanisms for pathway enzymes.

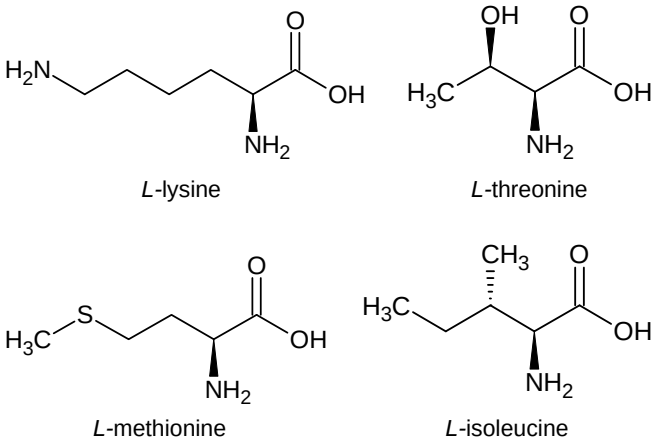

Figure 1. Structures of the four aspartate-derived amino acids.

BIOSYNTHESIS OF ASPARTATE SEMIALDEHYDE FROM ASPARTATE

Aspartate kinase (EC 2.7.2.4) and aspartate semialdehyde dehydrogenase (EC 1.2.1.11) catalyze the first two steps of the aspartate-derived amino acid pathway, prior to the branch-point at aspartate semialdehyde (Figure 3). At least five *A. thaliana* genes encode aspartate kinases. This relatively large number of enzymes catalyzing a single biosynthetic reaction may reflect the importance of aspartate kinase as a regulatory checkpoint in amino acid biosynthesis. Having multiple genes encoding this enzyme would allow more complex regulation at the level of transcription, translation, and allosteric interactions with both downstream metabolites and compounds from other plant biosynthetic pathways. Three of the *A. thaliana* aspartate kinase genes, At5g13280 (*AK1*), At5g14060 (*AK2*) and At3g02020 (*AK3*), encode monofunctional enzymes (Frankard et al., 1997; Tang et al., 1997; Yoshioka et al., 2001; Curien et al., 2007). The other two, At1g31230 (*AK-HSDH1*) and At4g19710 (*AK-HSDH2*), encode bifunctional enzymes with not only aspartate kinase, but also homoserine dehydrogenase (EC 1.1.1.3) activity (Ghislain et al., 1994; Paris et al., 2002; Rognes et al., 2002). Thus, these two bifunctional enzymes catalyze both the first and third steps in the biosynthesis of methionine, threonine, and isoleucine (Figure 2).

As the committing enzyme leading to the formation of lysine, methionine, threonine, isoleucine, and other downstream metabolites, aspartate kinase is subject to extensive allosteric regulation (Galili, 1995). The three monofunctional aspartate kinases are all subject to feedback inhibition by lysine. Additionally, AK1 is synergistically inhibited by *S*-adenosylmethionine, though *S*-adenosylmethionine by itself does not affect enzyme activity (Curien et al., 2007). Whereas lysine-dependent regulation is common in microorganisms, synergistic regulation by *S*-adenosylmethionine has only been described in *A. thaliana* and other plants (Rognes et al., 1980; Giovanelli et al., 1989). *A. thaliana* AK1 has been crystallized together with its two inhibitors (Mas-Droux et al., 2006b), suggesting a possible mechanism for feedback regulation. AK1 and other plant aspartate kinases contain two ACT domains (Pfam 01842), small regulatory motifs that are found in many proteins involved in amino acid metabolism (Chipman and Shaanan, 2001; Finn et al., 2008). The AK1 crystal structure reveals that both lysine and *S*-adenosylmethionine are bound to a single ACT domain, leading to the as yet untested hypothesis that structural changes induced by effector binding may make the modify ATP binding (Mas-Droux et al., 2006b). Allosteric inactivation of all three monofunctional aspartate kinases by lysine increases the apparent K_m for ATP and aspartate. This inhibition of enzyme activity restricts *A. thaliana* growth, making it possible to use lysine resistance as a mechanism to identify aspartate kinase mutants (Heremans and Jacobs, 1995, 1997).

A regulatory domain containing two ACT subdomains, which are the binding sites for allosteric effectors, separates the aspartate kinase and homoserine dehydrogenase portions of *A. thaliana* AK-HSD1 and AK-HSD2 (Paris et al., 2003). *In vitro* assays with cloned enzymes show that their activity is inhibited by threonine and leucine and activated by alanine, cysteine, isoleucine, serine, and valine (Paris et al., 2002; Rognes et al., 2002; Paris et al., 2003; Curien et al., 2005). Based on the concentrations of these amino acids in plant tissue, threonine and alanine are probably the most physiologically relevant effectors (Curien et al., 2005). Mutational analysis of the AK-HSD ACT domains showed two non-equivalent threonine binding sites. Binding of threonine to one site inhibits aspartate kinase activity and promotes binding of threonine to the second site, which inhibits homoserine dehydrogenase activity (Paris et al., 2003). Differential function in amino acid metabolism is suggested by the fact that AK-HSD1 and AK-HSD2 vary somewhat in their responses to the different effectors. In particular, physiological threonine concentrations inhibit threonine dehydrogenase activity of AK-HSD1 but not AK-HSD2 (Curien et al., 2005).

Aspartate semialdehyde dehydrogenase (EC 1.2.1.11), the other enzyme that is common to all three branches of the as-

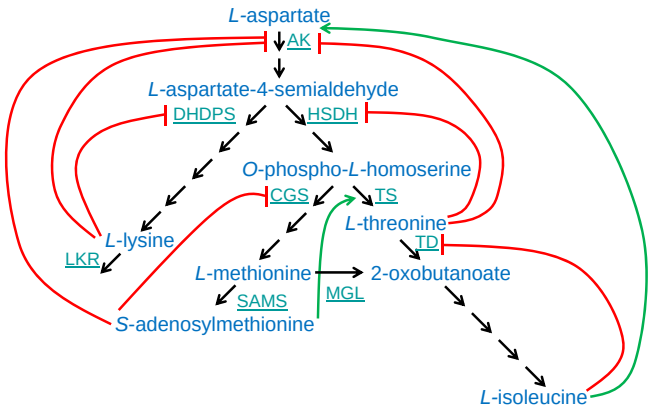

Figure 2. The aspartate-derived amino acid pathway. Key enzymes and metabolites are shown. Known allosteric regulation by compounds within the pathway is shown, activation with a green arrow and inhibition with a red bar. AK = aspartate kinase, DHDPS = dihydrodipicolinate synthase, HSDH = homoserine dehydrogenase, CGS = cystathionine γ-synthase, TS = threonine synthase, LKR = lysine-ketoglutarate reductase, TD = threonine deaminase, MGL = methionine γ-lyase, and SAMS = *S*-adenosylmethionine synthase.

partate pathway (Figure 2), is encoded by a single *A. thaliana* gene (At1g14810). Although Asd, the corresponding *Escherichia coli* enzyme, has been studied more extensively and the crystal structure has been determined (Hadfield et al., 1999), relatively little is known about the specific properties of plant enzyme. *In vitro* overproduction and analysis of the *A. thaliana* At1g14810 confirmed the predicted aspartate semialdehyde dehydrogenase activity (Paris et al., 2002). Unlike in the case of aspartate kinase, there is no confirmed allosteric regulation of aspartate semialdehyde dehydrogenase in *A. thaliana*. However, aspartate semialdehyde dehydrogenase extracted from maize callus and suspension cell cultures is inhibited by methionine and less so by lysine and threonine (Gengenbach et al., 1978).

LYSINE BIOSYNTHESIS

The aspartate-derived amino acid pathway branches at aspartate-4-semialdehyde (Figure 2), with dihydrodipicolinate synthase (DHDPS) (EC 4.2.1.52) catalyzing the first reaction leading to lysine biosynthesis (Figure 4). As the committing enzyme in an economically important amino acid biosynthesis pathway, DHDPS has been the subject of extensive research. Two *A. thaliana* genes, At3g60880 (*DHDPS1*) and At2g45440 (*DHDPS2*), encode dihydrodipicolinate synthases (Vauterin and Jacobs, 1994; Vauterin et al., 1999; Craciun et al., 2000; Sarrobert et al., 2000).

DHDPS in *A. thaliana* and other plants is feedback-inhibited by lysine and is the major regulatory checkpoint for lysine production (Vauterin et al., 2000; Galili, 2002). A feedback-insensitive aspartate kinase mutation causes increased threonine, but not lysine accumulation, suggesting that regulation of lysine biosynthesis occurs downstream of this enzyme (Heremans and Jacobs, 1995). In contrast, overexpression of feedback-insensitive DHDPS does cause a variable, though significant increase in lysine accumulation (Ben-Tzvi Tzchori et al., 1996). However, this lysine increase is tempered by a concomitant increase in lysine catabolism in developing seed (Zhu and Galili, 2003, 2004), which is described in more detail below. Substrate competition for

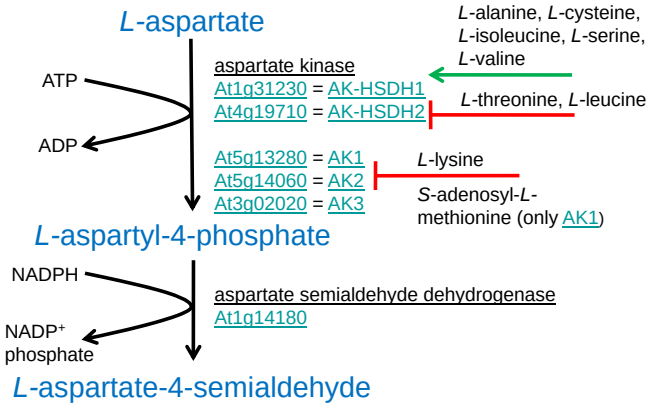

Figure 3. Biosynthesis of *L*-aspartate-4-semialdehyde from *L*-aspartate. These two enzymatic steps that are common to all branches of the aspartate-derived amino acid pathway. Known allosteric regulation is shown, activation with a green arrow and inhibition with a red bar.

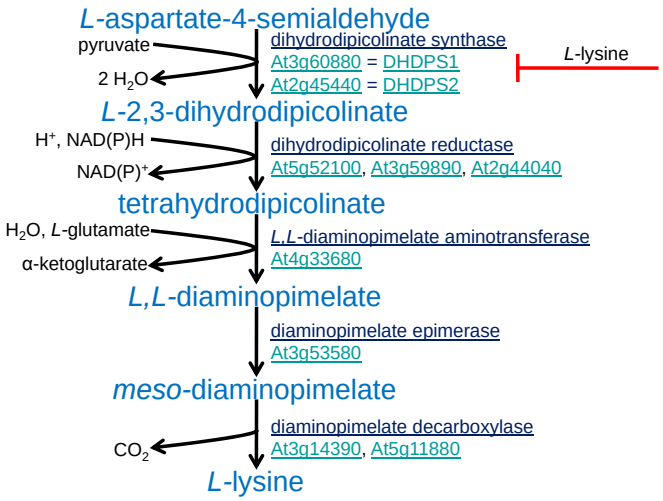

Figure 4. Lysine biosynthesis in *A. thaliana*. Enzymes involved in the biosynthesis of *L*-lysine from *L*-aspartate-4-semialdehyde are shown. Known allosteric inhibition is shown with a red bar.

aspartate-4-semialdehyde by DHDPS and homoserine dehydrogenase (Figure 2) is suggested by the fact that T-DNA insertions in *DHDPS2* cause reduced lysine synthesis and increased accumulation of threonine (Craciun et al., 2000; Sarrobert et al., 2000).

Other enzymes in the lysine biosynthetic pathway (Figure 4) have been studied less extensively than DHDPS. Genes encoding dihydrodipicolinate reductase (EC 1.3.1.26; At5g52100, At3g59890, and At2g44040), diaminopimelate epimerase (EC 5.1.1.7; At3g53580), and diaminopimelate decarboxylase (EC 4.1.1.20; At3g14390 and At5g11880) were identified based on sequence similarity to the respective bacterial enzymes (Hudson et al., 2005). Predicted activities of these enzymes were confirmed in the same study: Complementation of an *E. coli dapB* dihydrodipicolinate reductase mutant was successful with At3g59890, and At2g44040, but not At5g52100. Diaminopimelate epimerase activity of At3g53580 was confirmed by cloning and *in vitro* enzyme assays. Heterologous At3g14390 and At5g11880 expression rescued the lysine auxotrophy of an *E. coli lysA* mutant, showing that these genes both encode diaminopimelate decarboxylases.

Orthologs of bacterial enzymes normally required to convert tetrahydrodipicolinate into diaminopimelate are not present in the *A. thaliana* genome, suggesting that plants use a different path for this reaction (Hudson et al., 2005). This was confirmed by the identification a novel diaminopimelate aminotransferase enzyme (EC 2.6.1.83; At4g33680; Hudson et al., 2006). The At4g33680 gene was also discovered as the genetic basis of the defense-related *agd2* mutant, though the enzyme was miss-classified as a lysine transaminase (EC 2.6.1.36) in this study (Song et al., 2004). This incorrect enzyme identification may be due to the very high lysine concentrations that were used in these experiments, as well as the possible contamination of commercially available lysine stocks with *L,L*-diaminopimelate and/or *m*-diaminopimelate (Hudson et al., 2006).

The identification of a *Synechocystis* sp. functional ortholog of At4g33680 (Hudson et al., 2006) suggests that both higher plants

and cyanobacteria produce lysine *via* the pathway shown in Figure 4. Although most prokaryotes also synthesize lysine from *m*-diaminopimelate, the metabolic pathways leading from tetrahydrodipicolinate to *m*-diaminopimelate are distinct from the one found in *A. thaliana* (Hudson et al., 2006). Yet another, completely different pathway for lysine biosynthesis exists in *Saccharomyces cerevisiae* and most fungi, where *L*-2-diaminoadipate rather than *L*-aspartate-4-semialdehyde is the precursor for lysine biosynthesis (Velasco et al., 2002). Therefore, lysine is not an aspartate-derived amino acid in *S. cerevisiae.*

LYSINE CATABOLISM

A single *A. thaliana* gene, At4g33150, encodes enzymes that catalyze the first two enzymatic steps in lysine catabolism, lysine ketoglutarate reductase (EC 1.5.1.8) and saccharopine dehydrogenase (EC 1.5.1.9) (Figure 5). A role for catabolism in regulating lysine accumulation was confirmed by a T-DNA insertion in At4g33150, which increases lysine content in the seeds, but not in the rosette leaves of mutant plants (Zhu et al., 2001). At4g33150 is an unusually large *A. thaliana* gene, with a 3295 bp coding region and a total of 25 exons (Epelbaum et al., 1997). Interestingly, alternate transcription of this locus also produces both monofunctional enzymes, lysine ketoglutarate reductase and saccharopine dehydrogenase. A polyadenylation site located in an intron permits premature termination and the production of monofunctional lysine ketoglutarate reductase (Tang et al., 2002). Additionally, an internal promoter produces an alternate transcript that encodes a monofunctional saccharopine dehydrogenase (Tang et al., 2000). Transcripts from the At4g33150 locus are differentially regulated by both hormonal and metabolic stimuli, including jasmonate, abscisic acid, sugars, and nitrogen (Stepansky and Galili, 2003; Stepansky et al., 2005). The production of monofunctional enzymes with altered catalytic properties (Tang et al., 2000; Tang et al., 2002), as well as interactions between the two subunits of the bifunctional enzyme (Zhu et al., 2002), permits complex regulation of lysine degradation with a single genetic locus.

Expression of feedback-insensitive DHDPS in several plant species results in significantly increased lysine accumulation (Karchi et al., 1994; Falco et al., 1995; Mazur et al., 1999). However, in each case, the increase in seed lysine production is associated with increased catabolism by lysine ketoglutarate reductase. Therefore concomitant expression of feedback-insensitive DHDPS and reduction in the expression of lysine ketoglutarate reductase was predicted to cause a synergistic increase in free lysine content. This was confirmed in *A. thaliana*, where DHDPS overexpression and a T-DNA insertion in At4g33150 cause only 12-fold and 5-fold lysine increases, respectively, but a combination of the two genetic changes increases free lysine accumulation in the seeds 80-fold (Zhu and Galili, 2003). The negative growth effects of reduced lysine catabolism are alleviated when altered lysine synthesis and catabolism are restricted to the seeds using a phaseolin promoter (Zhu and Galili, 2004). This same approach, seed-specific regulation to increase synthesis and reduce catabolism, has been commercially implemented to increase lysine content in maize (Frizzi et al., 2008), showing a practical application to amino acid biosynthesis discoveries that were initially made in *A. thaliana*.

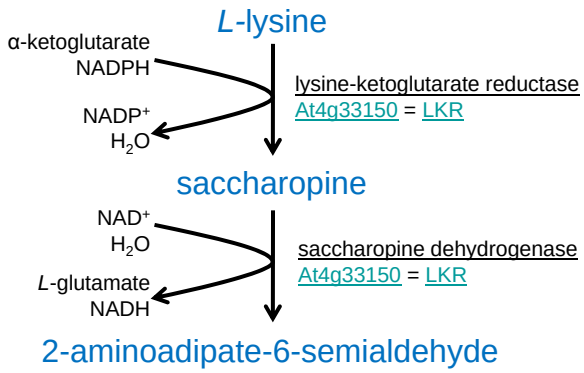

Figure 5. Lysine catabolism. A single bifunctional enzyme, lysine-ketoglutarate reductase - saccharopine dehydrogenase, catalyzes the first two steps of lysine catabolism in *A. thaliana*.

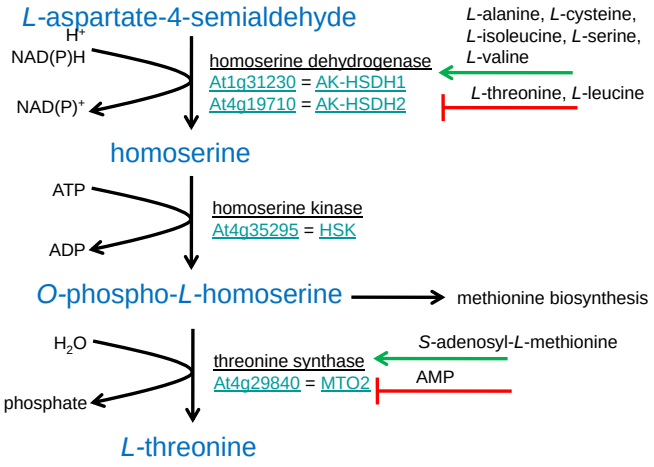

Figure 6. Threonine biosynthesis. Three enzymes catalyze the formation of *L*-threonine from *L*-aspartate-4-semialdehyde. Homoserine dehydrogenase and homoserine kinase are required for the biosynthesis of threonine and methionine. Known allosteric regulation is shown, activation with a green arrow and inhibition with a red bar.

Genes with sequence similarity to lysine decarboxylase (EC 4.1.1.18, *e.g.* At1g50575 and At5g26140), an enzyme that catalyzes the conversion of lysine to cadaverine, are found in the *A. thaliana* genome. However, to date, these enzymes have not been characterized in *A. thaliana*. If this enzymatic activity can be confirmed, it would represent an alternate pathway for lysine degradation in *A. thaliana*. Cadaverine formation from lysine has been reported from other plants. For instance, in pea seedlings inhibition of *S*-adenosylmethionine synthase and arginine decarboxylase by ethylene treatment causes increased lysine decarboxylase activity and cadaverine accumulation (Icekson et al., 1986). ALD1 (At2g13810), a lysine aminotransferase (EC 2.6.1.36) that was identified as a paralog of AGD2 (At4g33680; Song et al., 2004), may represent a third pathway for lysine degradation. In this case, the product would be piperidine-2-carboxylate or piperidine-3-

carboxylate, depending on which amino group is transferred from lysine. However, because *ALD1* is not a highly expressed gene in Arabidopsis, it is perhaps more likely that ALD1 functions to produce a pathogenesis-related signal rather than to degrade lysine.

THREONINE BIOSYNTHESIS

Homoserine dehydrogenase (EC 1.1.1.3) catalyzes the formation of homoserine from aspartate-4-semialdehyde as the first committing step in the pathway leading to the biosynthesis of threonine and methionine (Figure 2). The two bifunctional *A. thaliana* enzymes that catalyze the formation of homoserine, AK-HSDH1 and AK-HSDH2, have been described above due to their role as aspartate kinases that catalyze the first step in the aspartate-derived amino acid pathway. A single *A. thaliana* gene (At4g35295) encodes homoserine kinase (EC 2.7.1.39; Lee and Leustek, 1999), which converts homoserine to *O*-phosphohomoserine (Figure 6). In contrast to prior reports from pea and radish (Thoen et al., 1978; Baum et al., 1983), the *A. thaliana* enzyme is not allosterically inhibited by threonine, isoleucine, valine, or *S*-adenosylmethionine. Unless the substrate homoserine is added exogenously, overexpression of At4g35295 does not increase *O*-phosphohomoserine, threonine, or methionine accumulation in *A. thaliana* (Lee et al., 2005), showing that regulation of homoserine kinase activity is unlikely to have a major influence on pathway flux *in vivo.*

At4g29840 (*MTO2*) is the only *A. thaliana* gene that has been confirmed to encode threonine synthase (EC 4.2.3.1; Figure 6). This pyridoxal phosphate-dependent enzyme catalyzes the final reaction of threonine biosynthesis and is the first enzyme in the branch of the pathway leading to isoleucine (Figure 2). A second *A. thaliana* locus (At1g72810) has 81% amino acid sequence identity to At4g29840, but has yet to be confirmed to encode threonine synthase activity.

Cloned *A. thaliana* threonine synthase rescued the threonine auxotrophy of an *E. coli thrC* mutant (Curien et al., 1996). In contrast to the bacterial enzyme, threonine synthase from *A. thaliana* and other plants is allosterically activated by *S*-adenosylmethionine (Curien et al., 1998; Laber et al., 1999). To identify a mechanism for this allosteric activation, *A. thaliana* threonine synthase dimers have been crystallized with and without *S*-adenosylmethionine (Thomazeau et al., 2001; Mas-Droux et al., 2006a). Binding of *S*-adenosylmethionine causes a large conformational change in threonine synthase, which brings the pyridoxal phosphate cofactor into its active configuration in the enzyme. Other research has demonstrated that AMP acts as an inhibitor of *A. thaliana* threonine synthase activity *in vitro*, and both inhibition of enzyme activity by AMP and activation by *S*-adenosylmethionine are prevented when the first 77 amino acids of threonine synthase are deleted (Laber et al., 1999).

The position of *O*-phosphohomoserine as a branch-point metabolite in the biosynthesis of threonine and methionine suggests that regulation of threonine synthase by *S*-adenosylmethionine should play an important role in plant metabolite partitioning (Amir et al., 2002). Metabolic modeling and reconstitution of the pathway branch point with cloned enzymes is in agreement with this hypothesis (Curien et al., 2003). Whereas *S*-adenosylmethionine has a significant regulatory function, both *in vitro* enzyme assays and modeling suggest that enzyme inhibition by AMP probably does not play an important role under physiological conditions (Curien et al., 2003).

Further evidence for regulation of threonine and methionine biosynthesis at the *O*-phosphohomoserine branch-point comes from *A. thaliana* lines with elevated or decreased threonine synthase activity. Overproduction of ThrC, the *E. coli* threonine synthase, resulted in retarded seedling growth that could be alleviated by the exogenous addition of methionine (Lee et al., 2005), indicating competition between threonine synthase and cystathionine γ-synthase for a common substrate (Figure 2). The *mto2-1* mutant, which was isolated based on resistance to the toxic analog ethionine, has a 22-fold increase in free methionine content (Bartlem et al., 2000). Map-based cloning identified a missense mutation in the At4g29840 threonine synthase, which apparently reduces enzyme activity and thereby permits more metabolic flux towards the methionine branch of the pathway (Figure 2). Similar inhibition of threonine synthase activity with an antisense construct has been used in a practical application to increase the methionine content in potatoes (Zeh et al., 2001).

METHIONINE BIOSYNTHESIS

Although homoserine is the last common pathway intermediate for methionine and threonine biosynthesis in *E. coli* and most other microorganisms, plants use homoserine kinase (EC 2.7.1.39) to convert homoserine into *O*-phosphohomoserine, which then serves as a precursor for both threonine (Figure 6) and methionine (Figure 7) biosynthesis. Although the use of homoserine kinase in the methionine biosynthetic pathway is thought to be the ancestral state, *E. coli* and most other bacteria instead convert homoserine to *O*-succinylhomoserine and/or *O*-acetylhomoserine as the first step in the pathway (Gophna et al., 2005). The evolutionary causes of this difference between plant and bacterial methionine biosynthesis are not known. Perhaps the very active photosynthesis-associated metabolism in the plastids somehow makes *O*-phosphohomoserine a more preferable precursor for methionine biosynthesis in these organelles.

Molecular cloning, comparison to bacterial enzymes, and *in vitro* assays of enzyme activity have confirmed that the *A. thaliana* At3g01120 gene encodes cystathionine γ-synthase (EC 2.5.1.48; Figure 7), the committing enzyme for methionine biosynthesis (Kim and Leustek, 1996; Ravanel et al., 1998). Another *A. thaliana* locus, At1g33320, has 76% amino acid sequence identity to At3g01120, but there is as yet no confirmation that this gene encodes a cystathionine γ-synthase. Although *O*-phosphohomoserine is the most physiologically relevant substrate in *A. thaliana,* complementation of *E. coli* mutants showed that At3g01120 also can utilize *O*-succinylhomoserine and *O*-acetylhomoserine as substrates (Hacham et al., 2003).

Cystathionine γ-synthase competes with threonine synthase for a common substrate (Figure 2), and is therefore also a key regulatory point for the biosynthesis of threonine and methionine (Amir et al., 2002). Overexpression of cystathionine γ-synthase in *A. thaliana* causes a significant increase in the methionine and *S*-methylmethionine content, suggesting that this enzyme is a rate-limiting step in methionine biosynthesis (Kim et al., 2002). Addition of homoserine further increases methionine accumulation in

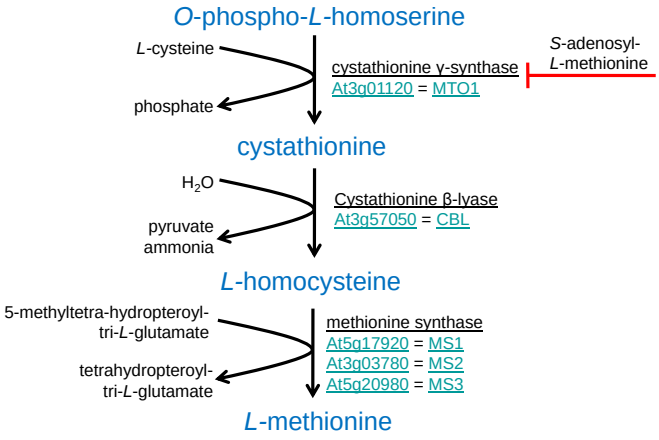

Figure 7. Methionine biosynthesis. Known inhibition is indicated with a red bar.

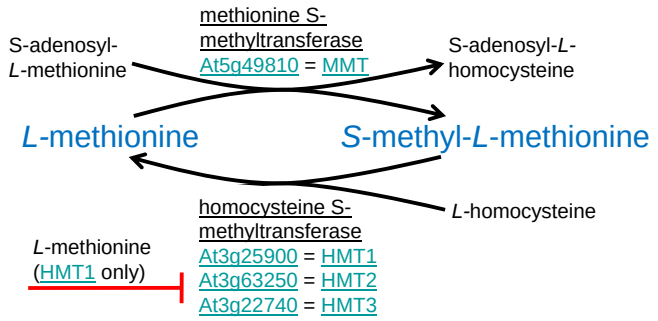

Figure 8. The *S*-methylmethionine cycle. Methionine *S*-methyltransferase and homocysteine *S*-methyltransferase interconvert *L*-methionine and *S*-methyl-*L*-methionine. Known inhibition is indicated with a red bar.

a cystathionine γ-synthase overexpressing line, an indication that the availability of *O*-phosphohomoserine limits methionine biosynthesis under these conditions (Lee et al., 2005). Conversely, expression of an At3g01120 antisense construct to reduce enzyme activity causes a 20-fold increase in *O*-phosphohomoserine and a small decrease in methionine accumulation (Gakière et al., 2000b; Kim and Leustek, 2000). These transgenic plants show severe growth abnormalities, which can be partially reversed by the exogenous addition of methionine.

A. thaliana mto1 methionine-overproducing mutant lines, which were isolated based on increased resistance to ethionine (Inaba et al., 1994), provided an inroad for extensive research on a novel mechanism for post-transcriptional regulation of cystathionine γ-synthase. Several independently isolated *mto1* point mutations all cause amino acid sequence changes in a 105 amino acid N-terminal region of cystathionine γ-synthase that is not found in bacterial enzymes (Chiba et al., 1999; Ominato et al., 2002). These missense mutations all compromise *cis*-acting regulation of the protein's own mRNA stability (Chiba et al., 1999; Suzuki et al., 2001). Additional mutational analysis of the At3g01120 N-terminus defined a regulatory domain of 11 to 13 amino acids that is highly conserved among plants and controls cystathionine γ-synthase mRNA stability (Ominato et al., 2002). Experiments with an *in vitro* translation system identified *S*-adenosylmethionine, rather than methionine, as the effector that binds to the N-terminus of cystathionine γ-synthase to reduce mRNA stability (Chiba et al., 2003; Onouchi et al., 2004). Binding of *S*-adenosylmethionine was also shown to cause translational arrest (Lambein et al., 2003; Onouchi et al., 2005), which precedes mRNA decay and is associated with the formation of a series of 5'-truncated mRNA products that may reflect the spacing of ribosomes on the mRNA (Haraguchi et al., 2008). Translational arrest, but not mRNA degradation in response to *S*-adenosylmethionine, also occurs when At3g01120 is expressed in a rabbit reticulocyte lysate system (Onouchi et al., 2008). Because animals do not have cystathionine γ-synthase, this shows that at least the translational arrest in response to *S*-adenosylmethionine occurs in the absence of other plant factors. Inhibition of *A. thaliana S*-adenosylmethionine synthase transcription by lysine may also indirectly increase cystathionine γ-synthase activity due to reduced *S*-adenosylmethionine levels (Hacham et al., 2007).

Transformation of tobacco with wildtype At3g01120, as well as with a version of the gene where the N-terminal domain was deleted, showed that only expression of the truncated protein is miss-regulated and greatly increases the abundance of methionine-derived volatile metabolites (Hacham et al., 2002). Interestingly, a transcript with an internal 90 nucleotide deletion that removes the regulatory domain near the N-terminus of cystathionine γ-synthase is expressed naturally in *A. thaliana* (Hacham et al., 2006). Unlike wild-type cystathionine γ-synthase, accumulation of the internally deleted protein is not affected by methionine addition, showing that the regulatory site has been deleted. Induction of folate deficiency by chemical inhibitors revealed yet another mechanism of cystathionine γ-synthase regulation, in this case a post-translational proteolytic removal of the N-terminal regulatory part of the protein (Loizeau et al., 2007). The crystal structure of tobacco cystathionine γ-synthase shows that the N-terminal domain is outside of the protein globule and probably accessible to proteases (Steegborn et al., 1999). Similar to the case of the internally deleted protein (Hacham et al., 2006), proteolytic removal of the regulatory domain increases cystathionine γ-synthase activity and thereby the production of methionine and *S*-adenosylmethionine (Loizeau et al., 2007).

Cystathionine β-lyase (EC 4.4.1.8), which catalyzes homocysteine formation as the second enzyme in the methionine biosynthetic pathway (Figure 7), has been studied less extensively than cystathionine γ-synthase. A single gene, At3g57050, encodes cystathionine β-lyase in *A. thaliana* (Ravanel et al., 1995). Analysis of recombinant At3g57050 produced in *E. coli* showed that the protein is a tetramer with physiological properties similar to those of bacterial cystathionine β-lyase enzymes (Ravanel et al., 1996). Cystathionine β-lyase overexpression does not significantly increase methionine accumulation in *A. thaliana* leaves (Gakière et al., 2000a). Nevertheless, antisense inhibition of cystathionine γ-synthase causes a two-fold increase in the abundance of cystathionine β-lyase (Gakière et al., 2000b), suggesting that there is

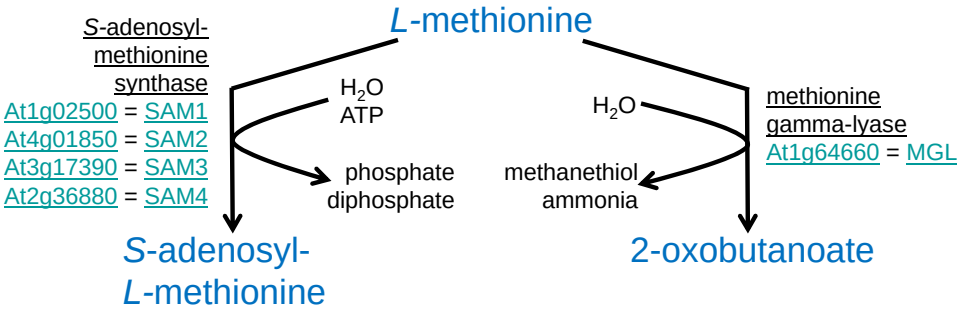

Figure 9. Methionine catabolism to *S*-adenosyl-*L*-methionine and 2-oxobutanoate.

at least some pathway flux regulation through altered transcription of this gene.

Methionine synthase (EC 2.1.1.14), which catalyzes the final reaction in the methionine biosynthesis pathway (Figure 7), is encoded by three genes in *A. thaliana,* At5g17920 (*MS1*), At3g03780 (*MS2*), and At5g20980 (*MS3*). Whereas MS1 and MS2 are cytosolic proteins, MS3 is localized to the chloroplasts (Ravanel et al., 2004). With the identification of MS3 as a plastid-localized methionine synthase, this confirms that the entire methionine biosynthetic pathway is contained in the plastids. MS1 and MS2 most likely function in the cytosol to regenerate methionine in the *S*-adenosylmethionine cycle.

THE *S*-METHYLMETHIONINE CYCLE

The enzymes homocysteine methyltransferase and methionine methyltransferase catalyze the interconversion of methionine and *S*-methylmethionine (Figure 8), a metabolic cycle that functions in phloem loading and amino transport (Bourgis et al., 1999; Lee et al., 2008). Whereas homocysteine methyltransferase forms two molecules of methionine from *S*-methylmethionine and homocysteine, methionine methyltransferase uses *S*-adenosylmethionine as a methyl group donor to form *S*-methylmethionine from methionine. Although homocysteine methyltransferase is also present in bacteria, fungi, and animals, methionine methyltransferase has been found only in plants (Ranocha et al., 2000). At5g49810, a non-essential gene encodes methionine methyltransferase (EC 2.1.1.12) in *A. thaliana* (Tagmount et al., 2002; Kocsis et al., 2003). Extensive biochemical characterization, including *in vitro* enzyme assays and complementation of *S. cerevisiae* and *E. coli* mutations (Ranocha et al., 2000; Ranocha et al., 2001), demonstrated that homocysteine methyltransferase (EC 2.1.1.10) is encoded by three *A. thaliana* genes, At3g25900 (*HMT1*), At3g63250 (*HMT2*), and At3g22740 (*HMT3*). Whereas HMT1 is feedback-inhibited by methionine, HMT2 and HMT3 are not. All three *A. thaliana* HMT enzymes use (*S,S*)-*S*-adenosylmethionine as a methyl group donor much less efficiently than *S*-methylmethionine (Ranocha et al., 2000; Ranocha et al., 2001). However, in natural systems, biologically active (*S,S*)-*S*-adenosylmethionine can spontaneously racemize at the sulfonium ion to form toxic (*R,S*)-*S*-adenosylmethionine (de la Haba et al., 1959), which is removed via a salvage pathway. Recent evidence of homocysteine methyltransferase activity specific for (*R,S*)-*S*-adenosylmethionine in *A. thaliana* (Vinci and Clarke, 2007) suggests that one or more of the three *A. thaliana* HMT enzymes may catalyze this reaction.

METHIONINE CATABOLISM

S-adenosylmethionine synthase (EC 2.5.1.6) directs about 80% of the metabolic flux of methionine to *S*-adenosylmethionine, which is used to methylate nucleic acids, proteins, lipids, and numerous other plant metabolites. After ATP, *S*-adenosylmethionine is probably the second-most frequently utilized co-factor in nature (Cantoni, 1975; Lu, 2000). The *S*-adenosylhomocysteine remaining from the methylation reaction is recycled to homocysteine and then methionine to complete the *S*-adenosylmethionine cycle. In addition, *S*-adenosylmethionine serves as a precursor for the biosynthesis of ethylene, polyamines, and other important plant metabolites. Four *A. thaliana* genes At1g02500 (*SAM1*), At4g01850 (*SAM2*), At3g17390 (*SAM3*, *MTO3*), and At2g36880 (*SAM4*) encode *S*-adenosylmethionine synthases (Peleman et al., 1989a; Peleman et al., 1989b; Shen et al., 2002).

Selection for *A. thaliana* resistance to ethionine identified *mto3* mutations, which increase methionine content up to 200-fold (Goto et al., 2002; Shen et al., 2002). Map-based cloning showed that the *MTO3* locus is the same as At3g17390, one of the four *A. thaliana S*-adenosylmethionine synthases, suggesting that the methionine content is caused by reduced flux from methionine to *S*-adenosylmethionine in the mutant background. Lignin, which requires several methylation reactions in its biosynthesis, was decreased by about 20% in the *mto3-1* mutant. The four *SAM* genes show considerable overlap in their expression patterns, and it is not yet known why the lack of SAM3 activity cannot be rescued by the other three S-adenosylmethionine synthases.

Both *S*-adenosylmethionine synthase transcription and enzyme activity in *A. thaliana* are reduced by exogenous lysine addition (Hacham et al., 2007). This, in turn, causes a significant reduction in the abundance of *S*-adenosylmethionine. Further experiments with the At1g02500 and At3g17390 *S*-adenosylmethionine synthases in an *in vitro* transcription/translation system showed reduced protein accumulation in the presence of lysine. Taken together, these results suggest that lysine or a downstream metabolite acts as a negative transcriptional regulator of *S*-adenosylmethionine synthases.

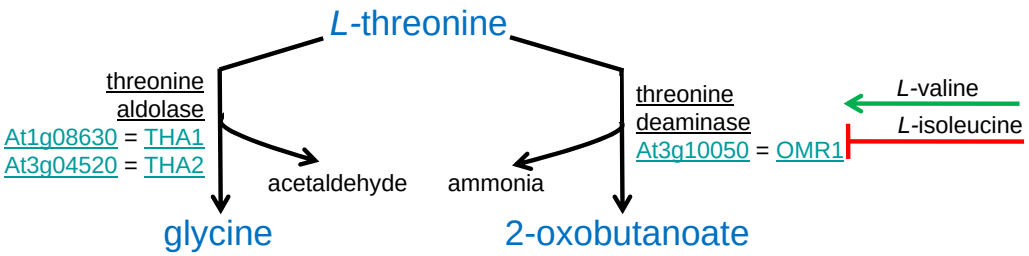

Figure 10. Threonine catabolism to glycine and 2-oxobutanoate. Known allosteric regulation is shown, activation with a green arrow and inhibition with a red bar.

An additional level of *S*-adenosylmethionine synthase regulation may come through *S*-nitrosylation, the covalent attachment of NO to cysteine residues of proteins, which affects several methionine-related enzymes in *A. thaliana* (Lindermayr et al., 2005). Incubation with the NO donor S-nitrosoglutathione results in reversible inhibition of SAM1, but not SAM2 or SAM4 (Lindermayr et al., 2006). Cysteine 114 in SAM1 was shown to be nitrosylated, and mutation of this residue to arginine greatly reduces the inhibitory effects of S-nitrosoglutathione. Since S-adenosylmethionine is a precursor for ethylene biosynthesis, inhibition of *S*-adenosylmethionine synthase by NO may mediate cross-talk between ethylene and NO signaling pathways in plants.

In another methionine catabolic reaction, methionine γ-lyase (EC 4.4.1.11) produces 2-oxobutanoate, methanethiol, and ammonia from methionine (Figure 9). A knockout mutation of At1g64660, the single *A. thaliana* gene encoding this enzyme, shows no visible phenotypes and has a ten-fold increase in free methionine under sulfate-limiting, but not normal growth conditions (Goyer et al., 2007). Methanethiol produced by methionine γ-lyase is incorporated into cysteine (Rebeille et al., 2006; Goyer et al., 2007). Additionally, labeling experiments with *A. thaliana* cell cultures show that the 2-oxobutanoate derived from methionine can serve as a precursor for isoleucine biosynthesis (Rebeille et al., 2006; Figure 2). Up-regulation of At1g64660 transcription during desiccation (Less and Galili, 2008), combined with the greatly increased isoleucine content of drought-stressed plants (Nambara et al., 1998), suggests the as yet unconfirmed hypothesis that biosynthesis of isoleucine from methionine is a response to drought stress in *A. thaliana.*

THREONINE CATABOLISM

Threonine deaminase (EC 4.3.1.19), which catalyzes the conversion of threonine to 2-oxobutanoate, is well-studied as the committing enzyme for isoleucine biosynthesis from threonine (Figures 10, 11). A single *A. thaliana* gene, At3g10050 (*OMR1*), encodes threonine deaminase activity (Mourad et al., 1995). Threonine deaminase from maize is inhibited *in vitro* by 2-(1-cyclohexen-3(*R*)-yl)-*S*-glycine, and growth inhibition of *A. thaliana* by this herbicide can be rescued with the exogenous addition of 2-oxobutanoate (Szamosi et al., 1994). Therefore, threonine deaminase is likely to be essential for *A. thaliana*, despite the fact that 2-oxobutanoate can also be produced form methionine by methionine γ-lyase (Figure 9).

Selection for *A. thaliana* mutants resistant to *O*-methylthreonine identified feedback-insensitive *omr1* mutations in threonine deaminase (Mourad et al., 1995). Changes in the feedback inhibition site of the enzyme made it resistant to inhibition by isoleucine and increased the free isoleucine content of the mutant plants. Comparison to the regulatory domain of *E. coli* threonine deaminase (IlvA) and site-directed mutagenesis of *A. thaliana* protein identified two separate sites of feedback inhibition (Wessel et al., 2000; Garcia and Mourad, 2004). Interaction of isoleucine with the binding sites induces conformational changes in threonine deaminase that lead to enzyme inhibition. Isoleucine binding to a high-affinity site promotes conformational changes that allow isoleucine binding to a low-affinity site, which in turn inhibits enzyme activity. In contrast, valine binding to the high-affinity site leads to different conformational changes, the release of isoleucine, and the reversal of threonine deaminase inhibition (Wessel et al., 2000). Binding of the effector molecules also influences the association of the threonine deaminase monomers. Whereas the native threonine deaminase is a tetramer, binding of isoleucine causes of less enzymatically active dimers, and tetramerization is restored by the addition of valine (Halgand et al., 2002).

In another *A. thaliana* threonine catabolic reaction, glycine and acetaldehyde are formed from threonine by threonine aldolase (EC 4.1.2.5; Figure 10). *In vitro* enzyme assays, as well as complementation of an *S. cerevisiae gly1 shm1 shm2* mutant, which is a glycine auxotroph (McNeil et al., 1994; Monschau et al., 1997), showed that two *A. thaliana* genes, At1g08630 (*THA1*) and At3g04520 (*THA2*) encode threonine aldolases (Jander et al., 2004; Joshi et al., 2006). Whereas *tha1* mutations greatly increase seed threonine content (Jander et al., 2004), *tha2* mutations cause a lethal albino phenotype (Joshi et al., 2006). Rescue of a *tha1 tha2* double mutant by overexpression of threonine deaminase (At3g10050) shows that glycine formation from threonine is not essential for *A. thaliana*, and that the lethal effects of *tha2* mutations may result from the accumulation of excess threonine. In addition to accumulating threonine, *tha1* seeds have ten-fold higher cysteine levels (Lu et al., 2008) suggesting as yet unknown regulation of cysteine metabolism by threonine, perhaps at the level of cystathionine γ-synthase (Figure 7). Since glycine can also be produced from serine and glyoxylate in plants, the functional importance of glycine production from threonine has not yet been determined.

Threonine dehydrogenase (EC 1.1.1.103) catalyzes threonine breakdown to 2-amino-3-oxobutanoate in animals and microbes (Epperly and Dekker, 1991; Edgar, 2002). However, although plant genes are occasionally annotated as “putative threonine dehydrogenase” based on sequence similarity, this enzymatic activity has not yet been confirmed in *A. thaliana* or other plants.

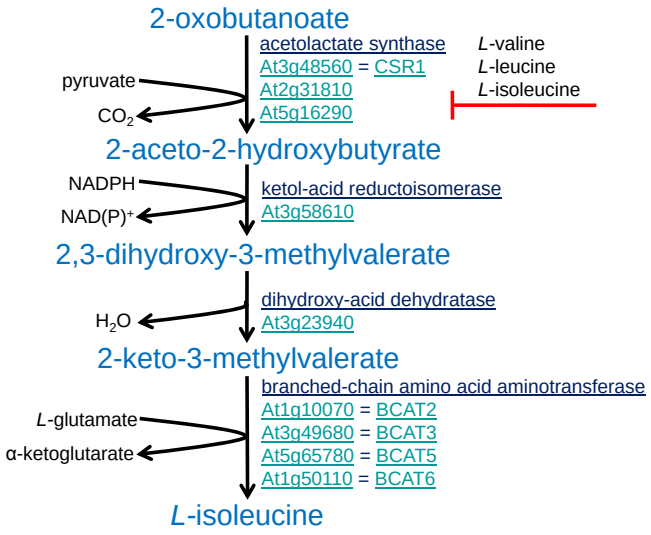

Figure 11. Isoleucine biosynthesis. Enzymes involved in the biosynthesis of *L*-isoleucine from 2-ketobutyrate. Known inhibition is indicated with a red bar.

ISOLEUCINE BIOSYNTHESIS

2-Oxobutanoate can be synthesized from both methionine and threonine in *A. thaliana* (Figures 9 and 10). However, based on herbicide studies that show lethal effects of threonine deaminase inhibition (Szamosi et al., 1994; Mourad et al., 1995), it seems likely that methionine γ-lyase plays a lesser role in isoleucine biosynthesis under normal growth conditions. Nevertheless, there may be some coordinated regulation of 2-oxobutanoate biosynthesis by these two enzymes. For instance, *A. thaliana* isocitrate lyase (EC 4.1.3.1; *ICL,* At3g21720) mutants cause 8-fold increased expression of threonine aldolase (*THA1*, At1g08630), which might make threonine less available for threonine deaminase, and could explain the resulting 50-fold increase in methionine γ-lyase (At1g64660) transcription in these mutants (Cornah et al., 2004).

Acetolactate synthase (EC 2.2.1.6) catalyzes the first step in the pathway from 2-oxobutanoate to isoleucine (Figure 11) and also the first step in the parallel biosynthetic pathway leading from pyruvate to valine and leucine (Coruzzi and Last, 2000). *A. thaliana* At3g48560 (*CSR1*) encodes the large, catalytic subunit of acetolactate synthase (Haughn and Somerville, 1990; Sathasivan et al., 1990; Chang and Duggleby, 1997), and At2g31810 encodes the small, regulatory subunit (Lee and Duggleby, 2001). Another gene, At5g16290, likely also encodes an acetolactate synthase small subunit, but has not yet been characterized. Whereas the large subunit by itself is insensitive to allosteric inhibition, combining the large and small subunits stimulates enzymatic activity that is sensitive to inhibition by valine, leucine, and isoleucine (Lee and Duggleby, 2001). Mutagenic analysis of the regulatory subunit showed two effector binding sites, one binding leucine and one binding valine or isoleucine (Lee and Duggleby, 2002). Selection for valine-resistant *A. thaliana* mutants resulted in acetolactate synthase activity that is less sensitive to inhibition by valine, leucine and isoleucine (Wu et al., 1994). However, resistance to synthetic herbicides is not altered in this mutant line.

Several studies have identified *A. thaliana* acetolactate synthase mutants with resistance to one or more of the sulfonylurea, triazolopyrimidine, imidazolinone, and pyrimidyl-oxo-benzoate herbicides (Haughn and Somerville, 1986, 1990; Sathasivan et al., 1990, 1991; Mourad and King, 1992; Mourad et al., 1993; Jander et al., 2003). So far, all of the identified mutations are in the large subunit of acetolactate synthase, At3g48560. Mutations conferring resistance to two different herbicides, chlorsulfuron and imidazolinone, can be combined into one enzyme to provide resistance to both herbicides simultaneously (Hattori et al., 1992; Mourad et al., 1994; Mourad et al., 1995). Some of the identified herbicide resistance mutations alter acetolactate synthase enzyme kinetics or binding of the cofactors FAD, Mg^{+} and thiamine diphosphate (Mourad et al., 1995; Chang and Duggleby, 1998). The crystal structure of *A. thaliana* acetolactate synthase with bound sulfonylurea or imidazolinone herbicides shows that these compounds do not bind at the active site itself, but rather block a protein channel leading to the active site (McCourt et al., 2006).

The next two enzymes in the isoleucine biosynthetic pathway, ketol-acid reductoisomerase (EC 1.1.1.86; At3g58610) and dihydroxy-acid dehydratase (EC 4.2.1.9; At3g23940) remain uncharacterized in *A. thaliana* (Binder et al., 2007). So far, genes encoding these enzymes have been identified and named based primarily on the similarity to those encoding ketol-acid reductoisomerase and dihydroxy-acid dehydratase in other species. The two enzymes likely catalyze the respective reactions in both of parallel pathways leading to the formation of isoleucine, valine, and leucine.

Seven *A. thaliana* loci, At1g10060 (*BCAT1*), At1g10070 (*BCAT2*), At3g49680 (*BCAT3*), At3g19710 (*BCAT4*), At5g65780 (*BCAT5*), At1g50110 (*BCAT6*), and At1g50090 (*BCAT7*) have sequence similarity to branched-chain amino acid aminotransferases (EC 2.6.1.42) from other organisms. Protein localization experiments show that these enzymes are targeted to different cellular compartments, the mitochondria (BCAT1), plastids (BCAT2, BCAT3, and BCAT5), and cytosol (BCAT4 and BCAT6), respectively (Diebold et al., 2002). With the exception of *BCAT4,* expression of the *A. thaliana BCAT* genes complements an *S. cerevisiae* bat1 bat2 double knockout mutant that lacks branched-chain amino acid aminotransferases activity (Diebold et al., 2002). BCAT4 has a different function in plant metabolism, contributing to methionine chain elongation during glucosinolate biosynthesis (Schuster et al., 2006). More recently, it was shown that the plastidic BCAT3 can act in both branched-chain amino acid and glucosinolate biosynthesis (Knill et al., 2008). *BCAT7* has not yet been investigated in detail, and there is no EST evidence showing that this gene is even transcribed.

In addition to their role in the biosynthetic pathway (Figure 11), branched-chain amino acid aminotransferases also catalyze the first step in valine, leucine, and isoleucine catabolism. BCAT1, which is localized to the mitochondria, was shown to initiate the catabolism of all three branched-chain amino acids (Schuster and Binder, 2005). Different sub-cellular localization of biosynthetic and catabolic BCAT enzymes may explain how this reaction can function in both directions in an individual plant cell.

Table 1. *A. thaliana* genetic loci and enzyme activities mentioned in this review

Gene name	AGI locus ID	Proven or predicted activity in *A. thaliana*	EC number
AK1	At5g13280	aspartate kinase	EC 2.7.2.4
AK2	At5g14060	aspartate kinase	EC 2.7.2.4
AK3	At3g02020	aspartate kinase	EC 2.7.2.4
AK-HSDH1	At1g31230	aspartate kinase	EC 2.7.2.4
		homoserine dehydrogenase	EC 1.1.1.3
AK-HSDH2	At4g19710	aspartate kinase	EC 2.7.2.4
		homoserine dehydrogenase	EC 1.1.1.3
	At1g14810	aspartate semialdehyde dehydrogenase	EC 1.2.1.11
DHDPS1	At3g60880	dihydrodipicolinate synthase	EC 4.2.1.52
DHDPS2	At2g45440	dihydrodipicolinate synthase	EC 4.2.1.52
	At5g52100	dihydrodipicolinate reductase	EC 1.3.1.26
	At3g59890	dihydrodipicolinate reductase	EC 1.3.1.26
	At2g44040	dihydrodipicolinate reductase	EC 1.3.1.26
AGD2	At4g33680	diaminopimelate aminotransferase	EC 2.6.1.83
	At3g53580	diaminopimelate epimerase	EC 5.1.1.7
	At3g14390	diaminopimelate decarboxylase	EC 4.1.1.20
	At5g11880	diaminopimelate decarboxylase	EC 4.1.1.20
LKR-SDH	At4g33150	lysine-ketoglutarate reductase	EC 1.5.1.8
		saccharopine dehydrogenase	EC 1.5.1.9
	At1g50575	lysine decarboxylase	EC 4.1.1.18
	At5g26140	lysine decarboxylase	EC 4.1.1.18
ALD1	At2g13810	lysine transaminase	EC 2.6.1.36
HSK	At4g35295	homoserine kinase	EC 2.7.1.39
MTO2	At4g29840	threonine synthase	EC 4.2.3.1
	At4g29840	threonine synthase	EC 4.2.3.1
MTO1	At3g01120	cystathionine γ-synthase	EC 2.5.1.48
CBL	At3g57050	cystathionine β-lyase	EC 4.4.1.8
MS1	At5g17920	methionine synthase	EC 2.1.1.14
MS2	At3g03780	methionine synthase	EC 2.1.1.14
MS3	At5g20980	methionine synthase	EC 2.1.1.14
MMT	At5g49810	methionine methyltransferase	EC 2.1.1.12
HMT1	At3g25900	homocysteine methyltransferase	EC 2.1.1.10
HMT2	At3g63250	homocysteine methyltransferase	EC 2.1.1.10
HMT3	At3g22740	homocysteine methyltransferase	EC 2.1.1.10
SAM1	At1g02500	S-adenosylmethionine synthase	EC 2.5.1.6
SAM2	At4g01850	S-adenosylmethionine synthase	EC 2.5.1.6
SAM3	At3g17390	S-adenosylmethionine synthase	EC 2.5.1.6
SAM4	At2g36880	S-adenosylmethionine synthase	EC 2.5.1.6
MGL	At1g64660	methionine γ-lyase	EC 4.4.1.11
THA1	At1g08630	threonine aldolase	EC 4.1.2.5
THA2	At3g04520	threonine aldolase	EC 4.1.2.5
OMR1	At3g10050	threonine deaminase	EC 4.3.1.19
CSR1	At3g48560	acetolactate synthase, catalytic subunit	EC 2.2.1.6
	At2g31810	acetolactate synthase, regulatory subunit	EC 2.2.1.6
	At5g16290	acetolactate synthase, regulatory subunit	EC 2.2.1.6
	At3g58610	ketol-acid reductoisomerase	EC 1.1.1.86
	At3g23940	dihydroxy-acid dehydratase	EC 4.2.1.9
BCAT1	At1g10060	branched chain amino acid aminotransferase	EC 2.6.1.42
BCAT2	At1g10070	branched chain amino acid aminotransferase	EC 2.6.1.42
BCAT3	At3g49680	branched chain amino acid aminotransferase	EC 2.6.1.42
		methionine-oxo-acid aminotransferase	EC 2.6.1.-
BCAT4	At3g19710	methionine-oxo-acid aminotransferase	EC 2.6.1.-
BCAT5	At5g65780	branched chain amino acid aminotransferase	EC 2.6.1.42
BCAT6	At1g50110	branched chain amino acid aminotransferase	EC 2.6.1.42
BCAT7	At1g50090	branched chain amino acid aminotransferase	EC 2.6.1.42

FUTURE PROSPECTS

With a few exceptions, some of which are noted above, almost all of the *A. thaliana* enzymes in the biosynthetic pathways of the aspartate-derived amino acids have been identified and characterized. Pathway enzymes and genetic loci mentioned in this review are summarized in Table 1. Several examples of allosteric regulation by downstream metabolites of the aspartate-derived amino acid pathway have been discovered (Figure 2). It is interesting that there are also numerous cases where seemingly unrelated amino acids and other metabolites regulate the activity of enzymes in the aspartate-derived amino acid pathway. Although the function of this regulation remains largely unknown, it may be a mechanism whereby plants can balance the metabolic flux through different biosynthetic pathways. Such cross-pathway regulation may also explain the seemingly unrelated amino acid changes that are often observed when expression or activity of aspartate-derived amino acid pathway enzymes is altered. The by now relatively well-characterized aspartate-derived amino acid pathway may serve as a model for future research to elucidate this complex, inter-pathway metabolic regulation

Transcriptional regulation is also still a largely uninvestigated aspect of amino acid biosynthesis. Global analysis of microarray data suggests that, unlike the extensive allosteric regulation of enzyme activity that occurs during amino acid biosynthesis, regulation of transcription may be more important during amino acid catabolism (Less and Galili, 2008). However, the presumed transcriptional regulators remain to be discovered. There is also undoubtedly regulation of biosynthetic enzymes in response to environmental stimuli and the amino acid needs of the plant. Complex transcriptional regulation is further suggested by the fact that homologous genes encoding the same enzymatic function often show very different expression patterns in various plant tissues and in the course of plant development. Future research will help to determine the underlying mechanisms of this transcriptional regulation of the aspartate-derived amino acid biosynthesis pathway in *A. thaliana*.

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